DIAGNOSTICS

The significance of voiding interval before testing urine samples for *Chlamydia trachomatis* in men

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Objective: To investigate the effect of time since last urination on chlamydial positivity rates in men.
Methods: Prospective study on men attending a genitourinary medicine clinic who were asked for the last time of their urination before obtaining their urine sample for testing for *Chlamydia trachomatis* with the Cobas Amplicor polymerase chain reaction (PCR) assay.
Results: Of the total of 1649 men studied, 621 (37.6% (95% CI 35.3% to 39.9%)) had a voiding interval of less than 2 hours. There was no statistical evidence of a difference in the positivity rate of chlamydial infection among men with a voiding interval of less than 2 hours (106/621, 17.1%) and that of men with a voiding interval of 2 hours or longer (170/1028, 16.5%); difference in proportions 0.5% (95% CI to 3.2% to 4.3%), p = 0.779.
Conclusion: Voiding interval does not have a significant impact on the performance of the Cobas Amplicor PCR assay in men.

The introduction into clinical practice of nucleic acid amplification tests (NAAT) with high sensitivity and specificity has significantly improved the diagnosis of patients infected with *Chlamydia trachomatis*.

One of the advantages of NAAT is their high performance when used on urine samples, which makes them ideal for screening programmes in non-clinical settings.[1]

According to the manufacturer's instructions for specimen collection before testing by the Cobas Amplicor *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) test the patient must not have urinated during the previous 2 hours. As a literature search did not find any publications on the time since last voiding urine and the performance of the Cobas Amplicor CT/NG test we inquired of the manufacturer (Roche) how this time interval was determined. Roche were also unable to find any papers on how the time elapsed between urine collection and the previous void affects the detection of chlamydia by their test. In addition, there are no ''in-house'' data relating to this. The selection of 2 hours as the minimum time between specimen collection and previous void dates back to before 1993 and was chosen during the development of the original Amplicor CT test, as this seemed a reasonable amount of time for the urethra to be repopulated with shedding cells. The test performance cited in the product insert is for samples where at least 2 hours elapsed (Brayshaw, molecular diagnostics sales manager, Roche Diagnostics, personal communication, 2004).

A subanalysis of a study on 23 men with chlamydial infection (including seven with less than 2 hours of voiding interval) did not show any change in the performance of ligase chain reaction on urine specimens of men with interval since last voiding of less than 2, 4, and 8 hours, respectively.[2]

As an interval of 2 hours since last voiding urine before providing a urine specimen for chlamydia testing may be a problem, and act as a barrier to testing, for a proportion of patients in clinical and non-clinical settings, it is important to investigate if this is a significant factor in the performance of NAAT.

The aim of this study was to investigate the effect of voiding interval on the performance of chlamydia PCR testing by examining the difference in the positivity rate of chlamydial infection between urine samples obtained less than 2 hours and those obtained 2 hours or longer after last urination.

METHODS

Patient recruitment and selection criteria

This was a prospective study on consecutive men who attended the Department of Genitourinary Medicine, Edinburgh, for chlamydia screening between February 2003 and January 2004. The chair of the Lothian Research Ethics Committee (LREC 02) confirmed that the study did not need ethical review.

All men were asked when they had last voided urine (less than 2 hours or 2 hours or more) before submitting their urine sample for chlamydia testing. Those who were not able to identify reliably the time of their last urination were not included in the study.

Information on patients' age, the volume of urine sample (whether <15 ml or $\geqslant 15$ ml) for chlamydia testing, and the interval since last voiding was recorded. The chlamydia results of patients were reviewed after 6 months of testing of the last patient.

Laboratory method

For the detection of chlamydia, a first voided specimen of urine was collected and sent to the laboratory within 4 hours. Urine specimens were stored overnight at 4°C before testing using the Cobas Amplicor CT polymerase chain reaction (PCR) test (Roche Diagnostics): all specimens were also tested with an internal PCR control. Negative specimens that tested negative with the internal PCR control assay were deemed inhibitory. The positive specimens were retested to confirm, and non-concordant results tested again. If the retest was negative the specimen was retested again and reported as positive or negative depending on the third result.

Statistical methods

We calculated differences with 95% confidence intervals (95% CI) in the percentages of men with positive tests for chlamydia according to voiding interval using Stata (Version 8.2, Stata Corporation, TX, USA).

Abbreviations: CT, *Chlamydia trachomatis*; EIA, enzyme immunoassay; FVU, first void urine; NG, *Neisseria gonorrhoeae*; NAATs, nucleic acid amplification tests; PCR, polymerase chain reaction

Table 1 Chlamydia positivity rate according to time since urination

Characteristic	Time since urination: Less than 2 hours Number positive	(%)	2 hours or more Number positive	(%)	Difference in proportions (95% CI)		p Value
All	106/621	(17.1)	170/1028	(16.5)	0.5	(−3.2 to 4.3)	0.779
Age group (years)							
15–19	12/59	(20.3)	19/81	(23.5)	−3.1	(−16.9 to 10.7)	0.661
20–24	45/192	(23.4)	76/311	(24.4)	−1.0	(−8.7 to 6.7)	0.799
25–29	23/124	(18.5)	32/203	(15.8)	2.8	(−5.7 to 11.3)	0.514
30–34	9/68	(13.2)	19/146	(13.0)	0.2	(−9.5 to 10.0)	0.964
35 or over	17/173	(9.8)	24/268	(9.0)	0.8	(−4.7 to 6.5)	0.758
Urine volume (ml)							
<15	17/81	(21.0)	23/139	(16.5)	4.4	(−6.4 to 15.2)	0.410
⩾15 or more	89/540	(16.5)	147/889	(16.5)	−.05	(−4.0 to 3.9)	0.979

RESULTS

During the study period, 3353 consecutive men were tested and 582 (17.4%, 95% CI 16.1% to 18.7%) had chlamydial infection. Of those, 1649 (49.2%, 95% CI 47.5% to 50.9%) consenting men gave reliable information on the voiding interval before submitting their urine samples for chlamydia testing with PCR. The main reason for such a discrepancy was patients' inability to reliably recollect the time of their last urination before submitting the urine samples for chlamydia testing. There was no statistical evidence of a difference in the chlamydia positivity rate between patients included (16.7%, 276/1649) and excluded (18.0%, 306/1704) from the analysis; difference in proportions −1.2% (95% CI −3.8% to 13.4%), p = 0.351.

Among study patients, the voiding interval was less than 2 hours in 621 (37.7%, 95% CI 35.5% to 40.0%) and 2 hours or more in 1028 (62.3%, 95% CI 64.0% to 64.7%. The age distribution, and the positivity rate of chlamydia infection in different age groups of men studied are summarised in table 1. Overall, there was no statistical evidence of a difference in the positivity rate of chlamydial infection among men with a voiding interval of less than 2 hours (106/621, 17.1%) and that of men with a voiding interval of 2 hours or longer (170/1028, 16.5%); difference in proportions 0.5% (95% CI −3.2% to 4.3%), p = 0.779. There was no statistical evidence of a difference in chlamydia positivity rates between specimens under 15 ml (18.2%, 40/220) or 15 ml and over (16.5%, 236/1429); difference in proportions 1.7% (−3.8% to 7.1%), p = 0.538.

DISCUSSION

A first void urine (FVU) is widely accepted as the specimen of choice for NAAT detection of chlamydia infection in men. However, individual NAATs have specific recommendations regarding the time that urine should be held before testing as well as the volume of urine that should be passed. For testing by the Cobas Amplicor CT/NG test, which is used in our laboratory, and many other centres, it is recommended that 10–50 ml of FVU urine should be collected after the individual has held their urine for at least 2 hours. As many patients being tested for chlamydia (38% in this study and 30% in a previous study[2]) have not held their urine for 2 hours it is important to determine the effect that this could have on the rate of positive tests.

We found no difference in the prevalence of chlamydial infection among men who, at the time of providing a urine sample for testing, reported an interval of less than 2 hours since last urination and those who had not urinated for 2 hours or more. This finding was consistent in all age groups, irrespective of the prevalence of chlamydia infection, which was highest in those aged 15–19 (22.1%) and lowest in those aged 35 or over (9.3%). These findings, based on 276 men with chlamydia, confirm the findings of a previous published study based on a subanalysis of 23 infected men and using the ligase chain reaction as test type.[2] There is a theoretical possibility that voiding interval, based on other than a 2 hour cut-off, might influence the performance of chlamydial PCR testing. However, an earlier study using three different enzyme immunoassays, and stratifying void time as <1 hour, 1–2 hours, 2–3 hours, and >3 hours found that longer void times did not improve sensitivity in 47 infected men.[3]

The volume of the urine sample may also be important in maximising the detection of chlamydia. In our study, chlamydia prevalence did not vary significantly between patients who provided a small volume of urine (<15 ml which is near the lower limit of the recommended volume) and those with a volume of urine (⩾15 ml). Small FVU urine volumes were not related to a shorter period since last voiding urine: the percentage of patients with a urine volume of <15 ml was 13% (81/621) in patients with an interval since urination of less than 2 hours, and 13.5% (139/1028) in patients with an interval of 2 hours or longer. We did not consider the possible effect of urine volumes near the upper recommended volume 50 ml. In practice, larger volumes are unlikely as the first void is normally made directly into a urine container with a maximum volume of approximately 20 ml. The importance of collecting a FVU was demonstrated in a study in which men collected three containers of urine (each containing 20–30 ml): using the ligase chain reaction the percentage of patients with a positive chlamydia test decreased from 11% (26/337) in the FVU to 8.4% (20/337) in the second voided urine.[2]

Care should be exerted in extrapolating our findings on voiding interval in men to urine specimens from women. We are unaware of any studies using NAATs, but an earlier study using enzyme immunoassay reported that an interval since last urination of more than 3 hours significantly reduced the performance of enzyme immunoassay (EIA) for detection of *C trachomatis* in urine from women.[4] The effect of the volume of the FVU from women has been studied using nucleic acid amplification tests. The Cobas Amplicor PCR performed equally well at all FVU volumes (⩽20 ml, 21–30 ml, 31–50 ml, and 51–90 ml), the ligase chain reaction performed less well at FVU volumes of ⩽20 ml, while transcription mediated amplification performed less well at volumes over 30 ml.[5]

Our study demonstrates that a voiding interval of less than 2 hours before providing urine for chlamydia testing does not have a significant impact on the performance of chlamydia PCR testing in men. Because urine is a widely used, and highly acceptable specimen for screening for chlamydia

Key messages

- Nucleic acid amplification tests allow high performance chlamydial testing on urine specimens
- The widely used Cobas Amplicor CT/NG test recommends that urine should be held for at least 2 hours before collecting a first void urine for chlamydia testing
- There was no significant difference in the positivity rate of chlamydial infection between men with a voiding interval of less than 2 hours and 2 hours or more
- There is no need for men to hold urine for 2 hours before providing a first void urine for chlamydia testing

infection in women,[6] it is important to demonstrate that a voiding interval of less than 2 hours before providing urine for chlamydia testing does not have a significant impact on the performance of chlamydia PCR testing in women.

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CONTRIBUTORS

KM, study design and data analysis; KM and HY both contributed to data collection, write up, and editing the paper.

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